

Time-Varying Magnetic Fields: Effect on DNA Synthesis

A. R. Liboff*, T. Williams, Jr., D. M. Strong, and R. Wistar, Jr.

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Abstract. Human fibroblasts have exhibited enhanced DNA synthesis when exposed to sinusoidally varying magnetic fields for a wide range of frequencies (15 hertz to 4 kilohertz) and amplitudes (2.3×10^{-6} to 5.6×10^{-4} tesla). This effect, which is at maximum during the middle of the S phase of the cell cycle, appears to be independent of the time derivative of the magnetic field, suggesting an underlying mechanism other than Faraday's law. The threshold is estimated to be between 0.5×10^{-5} and 2.5×10^{-5} tesla per second. These results bring into question the allegedly specific magnetic wave shapes now used in therapeutic devices for bone nonunion. The range of magnetic field amplitudes tested encompass the geomagnetic field, suggesting the possibility of mutagenic interactions directly arising from short-term changes in the earth's field.

Most studies of the effect of static magnetic fields on living systems have yielded negative or inconsistent results (1); the exception is studies of species that incorporate ferromagnetic materials as geomagnetic sensory elements (2). Experiments on time-varying magnetic fields have been fewer and more difficult to interpret (3). A therapeutic technique for accelerating repair in bone nonunions that subjects the site to a time-varying magnetic field (4) has achieved a measure of acceptance in the clinical community (5). However, the mechanism underlying this procedure is still largely unexplained, in part because the narrow and repetitive pulse shape of the wave

form, allegedly specific to the therapy, makes it difficult to perform and interpret in vitro cellular experiments (6).

We sought to determine the effect of sinusoidally varying magnetic fields on DNA synthesis in cell culture, particularly the frequency and intensity response, if any. A pair of matched Helmholtz coils (0.5 m, inside diameter; 0.25 m long), providing a horizontal magnetic field with an active field uniformity no less than 3 parts out of 17. Either could be an experimental or control (no field) unit. Periodically the roles were switched and, at other times, both were used simultaneously as controls. An au-

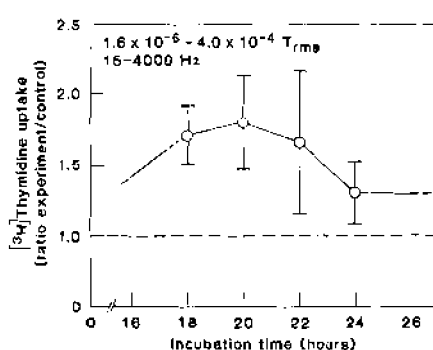
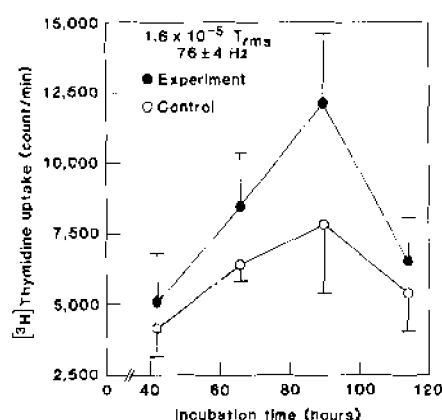


Fig. 1 (left). Tritiated thymidine uptake (mean counts per minute $\pm$ standard error) in human foreskin fibroblastic cells exposed to a magnetic field of amplitude 2.3×10^{-5} T oscillating at 76 ± 4 Hz in the horizontal plane (experiment) and uptake in unexposed cells (control). Mean levels differ at $P = 0.0001$, as determined from the pooled variances. The ambient 60-Hz magnetic field in the area of the incubators was $\approx 1 \times 10^{-7}$ T_{rms}. Note that the approximate intensity of the geomagnetic field is 5×10^{-5} T. Fig. 2 (right). Mean ratios and standard errors of [3 H]thymidine uptake in exposed cells to that in control cells for ten different combinations of frequencies and fields. The dotted line indicates the expected response if there is no effect due to a magnetic field. The peak at 20 hours corresponds to the middle of the S phase of the cell cycle. The dashed lines below 18 and above 24 hours represent the expected, but inexact trends, in these data.

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dio amplifier fed by a function generator supplied 0 to 1.0 root-mean-square ampere to either coil, enabling us to generate magnetic fields up to 1.0×10^{-3} root-mean-square tesla (T_{rms}) at frequencies ranging from 15 Hz to 20 kHz.

Two independent series of experiments were completed, both with human embryonic foreskin fibroblasts (7) as the test cell line.

In the first series, one frequency (76 ± 4 Hz) and one intensity ($1.6 \times 10^{-5} T_{rms}$) were used for all runs. Cells were seeded into eight 96-well culture plates (8) at densities of approximately 1.0×10^6 cells per milliliter, and the plates equally, but randomly, distributed between the control and experimental incubators. Following exposures of 24, 48, 72, and 96 hours, [3H]thymidine (9) was added to each well and the plates returned to their original incubators for 18 hours, after which all cells were washed twice in isotonic saline and collected on a filter strip by an automated sample harvester (10). The strips were dried, and the individual filter disks corresponding to each well were counted in a liquid scintillation counter. In 45 out of 48 paired plates, the amount of incorporated thymidine was higher for cells exposed to the magnetic field (Fig. 1).

In the second set of experiments, the cell cultures in the experimental incubator were subjected to various frequencies between 15 Hz and 4 kHz and to intensities between 1.6×10^{-6} and $4 \times 10^{-4} T_{rms}$. Tritiated thymidine was added to the cell medium at the time of seeding. The initial inoculation density was limited to 5×10^4 cells per milliliter. Paired (24-well) plates (11) were removed from both incubators at various times during a 24-hour period. Cell exposure was limited to 24 hours following inoculation to enhance cell synchrony. Selection of plates was not random as it was in the first experiment; seeding and harvesting of these cells were arranged beforehand. Corresponding wells in both plates were sequentially seeded, collected, and compared. In this manner, a fundamental data unit for a given frequency, intensity, and exposure time consisted of the ratio of thymidine uptake in an experimental well to that for a matched control. Averages of these units for all such pairs provided uptake ratios. Preliminary trials indicated that a peak in this ratio occurred after about 20 hours of exposure. Subsequent data collection was then restricted to four exposure times: 18, 20, 22, and 24 hours (Fig. 2).

In separate experiments to determine cell growth as a function of time, the peak in uptake was found to correspond

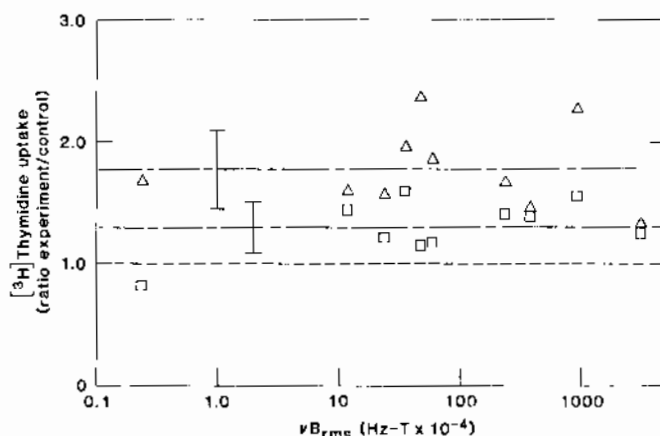


Fig. 3. The mean ratios (from Fig. 2) as a function of the product of frequency and magnetic field intensity. Exposure times of 20 hours (Δ) and 24 hours ($\square$) are shown. Note the apparent lack of variation in response for four orders of magnitude, indicating that this interaction is independent of the time derivative of magnetic field, $\partial B/\partial t$. The two upper lines represent the mean levels for the 20- and 24-hour exposures, respectively.

to the middle of the S phase of the cell cycle. Although the enhancement of thymidine uptake was, on average, no greater than 80 percent (Fig. 2), ratios two and three times this occasionally resulted in individual experiments. Figure 3 shows, for two separate exposure times, the relative uptake as a function of the product of frequency ν and root-mean-square field strength B (B_{rms}): this product is directly proportional to the time derivative of the magnetic field. As such, the slopes of the lines plotted will test whether Faraday's law is implicated in the results.

These experiments show that there is an interaction, albeit a subtle one (the results from Fig. 2 represent approximately 100 trials), between time-varying magnetic fields and DNA synthesis. Inasmuch as our fields varied sinusoidally, some measure of doubt is introduced as to whether the particular wave shape now used to accelerate bone fusion is indeed as necessary as has been claimed. An alternative, though not a likely one, is that two separate cellular interactions occur at low-frequency magnetic fields.

Although it is reasonable to suggest that Faraday's law is involved in inducing extracellular and intracellular currents according to $\nabla \times E = -\partial B/\partial t$ (where E is the induced electric field and t the time), it should then follow that the observed response will scale as $|\partial B/\partial t| = 2\pi\nu B$. The lack of such a response (Fig. 3) may mean that induced eddy currents do not play a role in this phenomenon, or it may indicate that the effect is a saturable phenomenon—for example, either a configurational effect or a self-limiting shift in the onset of the S phase.

Our most interesting result relates to the magnitude of the magnetic intensities that were studied. The lowest level of field strength that we used is at or below

the intensity of the geomagnetic field (approximately $5 \times 10^{-5} T$). In surveying the experimental area, it was found (12) that the 60-Hz ambient field is only two orders of magnitude lower. We estimate the threshold for this effect to be $\nu B_{rms} \approx 5$ to $25 \mu T/sec$, close to the value reported ($\sim 10 \mu T/sec$) (13) as interfering with normal chick embryo development. Therefore, care should be taken when studying cell culture that there are no perturbations from local 60-Hz magnetic fields, such as those resulting from fans, motors, and fluorescent lights in close proximity to the culture.

Finally, we can also remark on the potentially mutagenic response that may accompany this phenomenon, if indeed chromosome replication is affected. This would constitute a new type of mutational force and perhaps could be used to explain, in a rather direct fashion, the interrupted speciation accompanying geomagnetic reversals (14).

A. R. LIBOFF*
T. WILLIAMS, JR.
D. M. STRONG
R. WISTAR, JR.

Naval Medical Research Institute,
Bethesda, Maryland 20814

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* Present address: Department of Physics, Oakland University, Rochester, Minn.

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